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AD_____

GRANT NUMBER DAMD17-94-J-4308

TITLE: Identification and Characterization of Molecular Abnormalities of 11p Genes in Human Breast Cancer

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REPORT DATE: October 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1996	Annual (1 Oct 95 - 30 Sep 96)

4. TITLE AND SUBTITLE

Identification and Characterization of Molecular Abnormalities of 11p Genes in Human Breast Cancer

5. FUNDING NUMBERS

DAMD17-94-J-4308

6. AUTHOR(S)

Feinberg, Andrew P., M.D., M.P.H.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Johns Hopkins University of Medicine
Baltimore, Maryland 21205-4196

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution authorized to U.S. Government agencies only (proprietary information, Apr 97). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS

Oncogenes, Loss of Heterozygosity, Genomic Imprinting, Tumor Suppressor Genes, Molecular Cloning, Humans, Anatomical Samples, Breast Cancer

15. NUMBER OF PAGES

23

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

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Andrew Feeley 4/24/97

PI - Signature Date

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INTRODUCTION

This project is based on the hypothesis that at least one tumor suppressor gene on chromosomal band 11p15 is involved in breast cancer progression (1-3). This hypothesis is based on the fact that loss of heterozygosity (LOH) of 11p15 occurs in at least half of breast cancers that metastasize, and that a normal chromosome 11 suppresses tumorigenicity of MCF-7 breast cancer cells (4). Further support for a role of 11p15 is the fact that normal mammary epithelial cells lose 11p15 when immortalized by Simian Virus 40 (SV40) DNA (5).

At the beginning of this work, little was known about the precise location of such a tumor suppressor gene within the >10 Mb that comprise this region. Furthermore, breast cancer involves multiple genetic alterations on several chromosomes (6,7), and 11p15 is also involved in other tumor types (8,9). LOH and genetic complementation experiments suggest the presence of multiple tumor suppressor genes, even on this chromosomal band (9-12).

The purpose of these studies is to localize and identify an 11p15 breast cancer gene. We are taking two approaches to this work. First, we are using the classical indirect approach of analyzing polymorphic markers from 11p15 to map the region of LOH in breast cancers. Second, we are employing a novel direct strategy that we developed to transfer subchromosomal transferable fragments, or STF's (10), into breast cancer cells, in order to identify functionally the location of a breast tumor suppressor gene. As described below, we have made progress toward both of these goals.

We also had originally planned to investigate loss of imprinting (LOI) in breast cancer, a novel type of epigenetic alteration in cancer that we discovered, that leads to increased expression of insulin-like growth factor II (IGF2), an important autocrine growth factor in breast cancer (13-17). In the last project period, we noted that work in the original proposal to investigate loss of imprinting in breast cancer could not be pursued because of a reduction in budget at the time of the award. Deletion of this aim from the grant was approved by the Army, and we sought funding for that work from an RFA for an administrative supplement to the National Action Plan on Breast Cancer (NAPBC). That proposal received a most favorable peer-review priority rating (125) but was not funded. We have therefore performed some of that work at reduced effort under the auspices of this proposal, while continuing to pursue alternative funding for it. As described below, this work has been most productive, even though we could devote only a limited effort to it.

BODY OF REPORT

1. Experimental Methods

Isolation of Genetic Markers: Yeast artificial chromosome (YAC) and cosmid clones were isolated as previously described (18). Bacterial artificial chromosome (BAC) clones (Genetic Research Inc.) and P1 artificial chromosome (PAC) clones (Genome System Inc.) were isolated using a polymerase chain reaction (PCR) based screening method, and P1 clones were isolated by hybridization of high density filters (Genome Systems Inc.) using single copy probes. All BAC, PAC, and P1 DNAs were isolated using alkaline lysis, followed by phenol/chloroform extraction. Novel polymorphic markers were identified by hybridization of genomic DNA digests with cosmid subclones, or by hybridization of subclones with a dinucleotide repeat probe.

Exon Trapping: Exon trapping was performed using the pSPL3B vector (Paul Nisson, BRL, personal communication; ref. 19). Individual BAC, PAC, or P1 clones, or cosmid pools, were digested with Bam H1 and Bgl II and shotgun cloned into pSPL3B. Exons were sequenced with the SD2 primer (BRL) using an ABI377 automatic sequencer. Unique exons were mapped to their original genomic clones using PCR and hybridization.

Isolation of DNA and RNA from Tissues: Breast cancer tissues were obtained from the Johns Hopkins Hospital Pathology Department, from M. Luce (Roche Biomedical Diagnostics), and from the Cooperative Human Tissue Network, after written approval by institutional committees for compliance with all applicable regulations. Specimens were maintained at -135°C until use. For DNA isolation, tissues were pulverized in liquid nitrogen and suspended in TE9 (0.5M Tris Hcl, pH 9.0, 20 mM EDTA, 10 mM NaCl), digested with proteinase K (0.2 mg/ml) in the presence of 1% SDS at 50°C overnight, purified by phenol/chloroform extraction and precipitated with sodium acetate/ethanol. RNA was isolated using RNAzol B (TEL-TEST, Inc.).

PCR and Southern and Northern Hybridization: PCR was performed using primers designed by sequencing plasmid subclones containing dinucleotide repeats. The reactions were carried out on a Robocycler (Stratagene) under conditions tailored to each primer set using a commercial kit (Opti-Primer, Stratagene). Southern hybridization was performed by the method of Church and Gilbert (20), using plasmids or cosmids labeled by the method of Feinberg and Vogelstein (21). Northern hybridization was performed as described (23).

Microcell Transfer of Subchromosomal Transferable Fragments (STF's): STF's were prepared as described (10). MCF-7 and HuMI cells were cultured in DMEM/10% fetal calf serum. A9 cells carrying STF's were cultured for 48 hours in cytochalasin B and G418 (400 μg/ml). Microcells were prepared by culturing STF-containing donor cells in colcemid (0.5 μg/ml) for 48 hours, centrifuging in cytochalasin B (10 μg/ml), and filtering, and then fused to recipient tumor cells with PEG as described (22).

RNase Protection Assay: PCR-generated gene fragments were subcloned into pBluescript II KS +/- (Stratagene) for generation of antisense RNA probes, using the Ambion MAXIscript In Vitro Transcription kit and α-^{32}P-UTP according to the manufacturer's directions. 1 μg of pre-purified pTRI-GAPDH (Ambion, Inc.) was also used to generate antisense glyceraldehyde 3-phosphate dehydrogenase (GAPDH) transcripts for quantitation controls. RNase protection assays (RPA) were carried out utilizing the RPA II Kit (Ambion, Inc.) with approximately 20 μl of poly(A+) mRNA per reaction.

Allele-Specific Gene Expression: 0.5 μg of RNA was treated with DNase I (Boehringer Mannheim), heated to 95°C for 7 minutes, and reverse transcription (RT)-PCR was performed for IGF2 and H19 as described (24). Each sample was analyzed in duplicate in the presence and absence of reverse transcriptase to exclude DNA contamination. Gels were blotted and hybridized with a ^{32}P-labeled internal specific oligonucleotide probe (24), and quantified with a PhosphorImager (Molecular Dynamics).

2. Results

Task 1, LOH Mapping of 11p15: The purpose of these experiments was to determine where within 11p15 are the common regions of overlap of LOH in breast cancer, which will

more narrowly define the interval containing candidate tumor suppressor genes. We have now analyzed 50 paired breast tumor and normal samples, using 12 highly informative microsatellite markers. These data were analyzed in two different ways. Most published reports score partial LOH, or allelic imbalance, with as little as 15% reduction of intensity of one of the bands representing the polymorphism. When we scored tumors by this criterion, we found two regions of LOH, extending telomeric from D11S1318 to the end of the chromosome, and centromeric from D11S1318 (Fig. 1 below), similar to that described by Cavenee and colleagues (11). The maximum frequency of LOH was at D11S860, representing 30% of Stage 3 and 4 tumors. However, these experiments were not particularly helpful in sublocalizing allelic loss to smaller intervals, in that it is difficult to place a boundary of LOH using quantitative criteria. The second approach was to score allelic loss by a much more stringent criterion, >95% loss, in which we found only 10% of tumors showing LOH by this criterion, but the data were more reliable in interpreting boundaries of allelic loss. One tumor in particular showed LOH for D11S4146 but not for D11S860. Combined with other data from our and other laboratories, this would localize a tumor suppressor gene to the interval D11S860 to tyrosine hydroxylase (TH), representing only approximately 2 Mb (Fig. 1). This is the narrowest interval that has yet been defined for breast cancer LOH in this interval. The data are at variance with a recent report by Tran and Newsham (12), which excluded the very interval we have identified. However, those authors used a very relaxed criterion for scoring LOH, which could account for the conflicting result. As discussed below, we are in the process of cloning a portion of most or all of the genes in the interval we have identified. These experiments are also providing an additional valuable reagent. by hybridizing subclones with a dinucleotide repeat probe, we are identifying novel highly polymorphic markers suitable for PCR analysis.

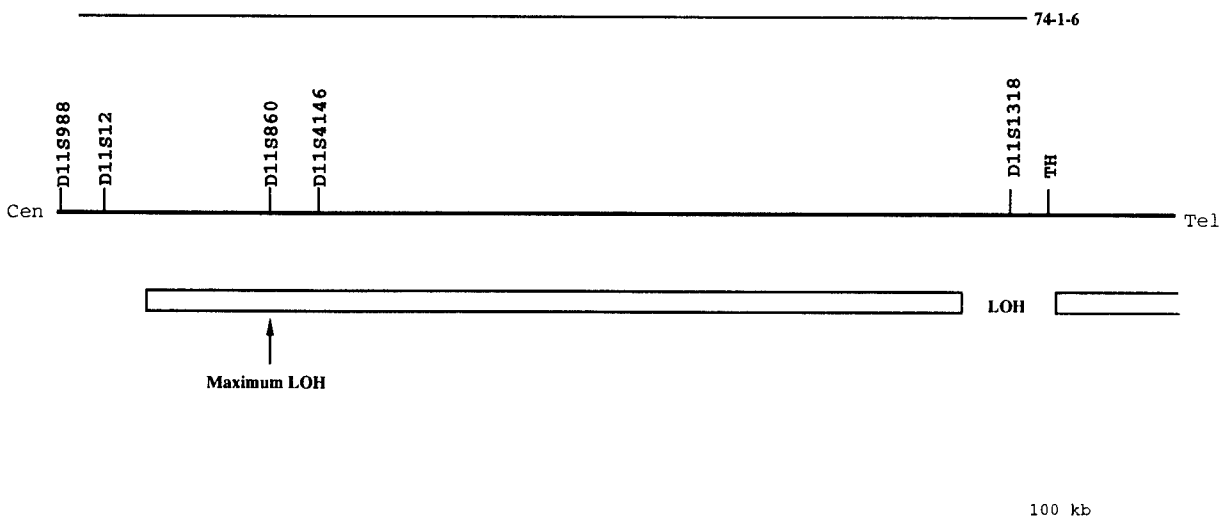

Fig. 1. LOH mapping of 11p15 breast tumor suppressor gene. Rectangles show minimal regions of complete LOH. The arrow shows the region of maximal partial LOH.

In addition, as discussed below, we are designing PCR primers at approximately 12 kb intervals, representing individual exons throughout this interval, so that we can look for homozygous deletions with much greater saturation of the interval. Our current markers (average interval 100 kb) have not revealed homozygous deletions, but we are hopeful that the additional probes will be more revealing. Finally, we will analyze an additional set of 100 paired samples from paraffin blocks. In this manner, we will be able to focus on metastatic tumors, which are more likely to show 11p15 loss. This is important, because primary tumors have shown karyotypic heterogeneity (24), and since tumors that metastasize tend to show 11p15 loss, the metastases themselves should be enriched for those cells. We are also able to enrich subportions of the tumors essentially to homogeneity. Our approach, which we term "microallelotyping", allows analysis of small portions of the tumors is less heterogeneous than randomly chosen samples (25).

Task 2, Functional Localization of an 11p15 Gene: The purpose of these experiments was to transfer subchromosomal transferable fragments (STF's), containing varying portions of 11p15, into breast cancer cells, in order to determine directly which of these contains a gene capable of suppressing tumor cell growth. As described in the previous report, while we were able to introduce STF's into tumor cells, we were not able to propagate them to sufficient numbers for in vivo tumorigenicity assays. While one could argue that the cells are showing in vitro growth arrest, that is not the phenotype seen when a whole chromosome 11 is introduced into breast tumor cells (4), and we felt that it would be unwise to conclude that such fragments contained a tumor suppressor gene based on in vitro data. We have now been able to overcome the problem of in vitro growth of STF's in MCF-7 cells, by propagating the cells at moderate to high density. We do this by trypsinizing individual small colonies once they are identified after transfer, and replating them in 96-well microtiter plates, in order to keep them at relatively high density, then transferring to 24-well plates, and finally 25 cm^2 dishes. While this task has taken longer than originally anticipated, we are now introducing STF-containing tumor cells into nude mice, so that we can complete these important assays.

Task 3, Identification of Candidate Breast Cancer Genes: As described above, to date, using known markers, we have sublocalized by LOH mapping one region of LOH between D11S4146 and TH. We are hopeful that the additional polymorphic markers we are identifying in this interval will allow more precise mapping of smaller deletions that were previously undetectable.

However, the experiments in this task are not entirely dependent upon LOH and homozygous deletion mapping. We have thus embarked on an effort to clone at least a portion of all of the genes within the more telomeric interval. We screened PAC, BAC, and P1 libraries using probes derived from the cosmids we had already mapped to this interval, as well as known STS's. We then derived end clones from these large insert clones, and re-screened the libraries in order to generate a complete genomic contig encompassing this approximately 2 Mb interval, with two residual gaps, each <100 kb. We then performed exon trapping through this interval, identifying 165 individual exons, corresponding to one exon per 12 kb, representing relatively complete saturation. Many of these exons corresponded to expressed sequence tags (EST's), or random sequences deposited in GenBank. The cDNA's corresponding to these sequences were then simply requisitioned from the appropriate depository. In addition, we have developed a rapid PCR-based procedure for isolating cDNA's from a library in only 2 days, that is 100% sensitive if preliminary experiments show that the cDNA is represented in the library, and 90%

specific, based on sequencing of the isolated clones. In this manner, we have already isolated 23 cDNA's corresponding to 51 of the exons. Some of these correspond to known genes, whose homologues in humans had not yet been obtained or mapped. Others correspond to novel genes. We estimate that all of the cDNA's in this interval will be isolated within 6 months. Please refer to the figure below for localization of these cDNA's (Fig. 2).

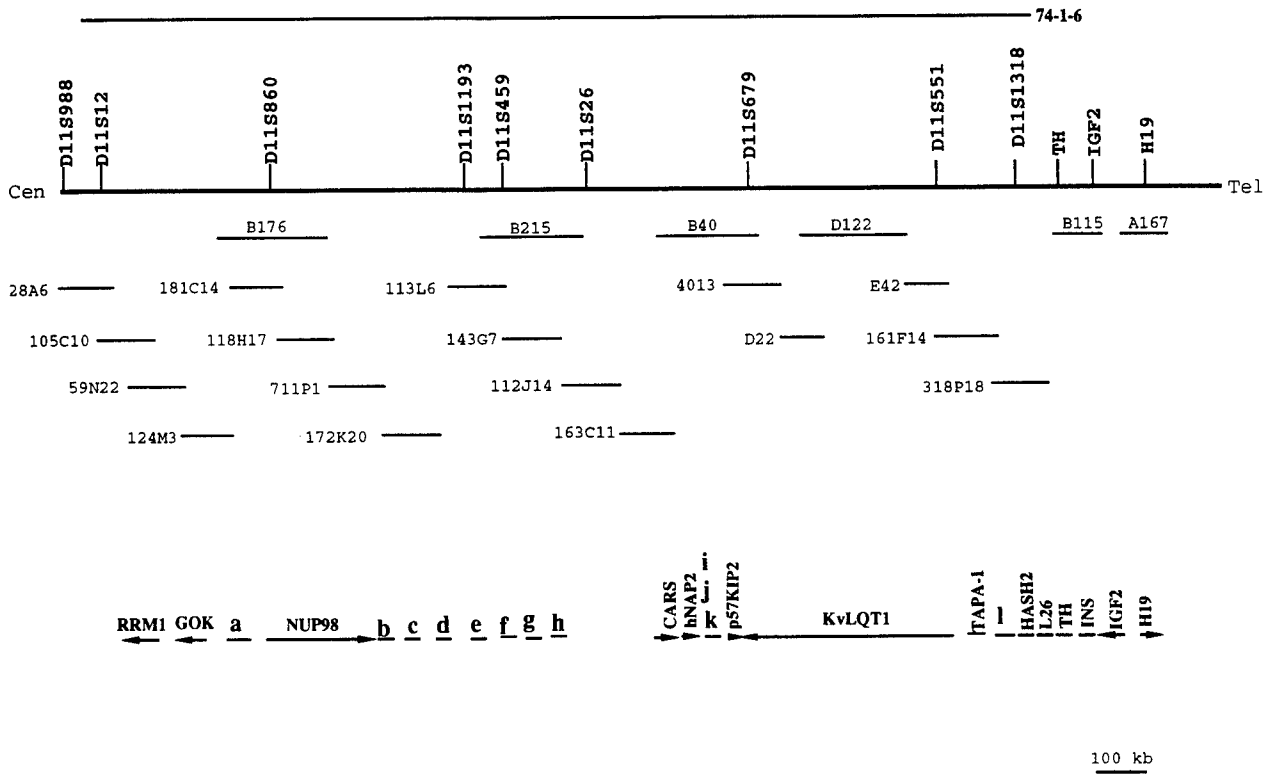

Figure 2. Genomic contig of 11p15 and identification of candidate breast tumor suppressor genes. cen=centromere. tel=telomere.

Sequence analysis is of some value in identifying genes more likely to be involved in breast cancer. However, such estimates can be misleading, so we are also using the exons themselves to screen breast cancers for homozygous deletions, using 50 paired tumor-normal specimens and 200 tumor-only specimens already obtained. The latter are suitable for screening for homozygous deletions but not for LOH, because of the lack of normal tissue.

In addition, we are analyzing breast cancers for mutations in genes that we identify that are involved in other types of malignancies. For example, as discussed below, we and our collaborators have found rearrangement of the nucleoporin gene NUP98 on 11p15 in leukemia (26). We also mapped the cyclin-dependent kinase inhibitor gene $p57^{KIP2}$ to this interval (18), we found that it is normally imprinted (27), and we have found abnormal imprinting and epigenetic silencing of the gene in Wilms' tumor (28). Thus, we are also looking for involvement of these genes in breast cancer.

Task 4, Characterization of Candidate Breast Cancer Genes: The purpose of this task was to characterize candidate genes identified in Tasks 1-3, by Southern and Northern

hybridization, mutational analysis in tumors, and, if indicated, genetic complementation. In the previous reporting period, we identified two novel candidate genes.

The first of these was NUP98, a nucleoporin which, in collaboration with the laboratory of N. Copeland, we found to be rearranged in two patients with leukemia involving an 11p15 translocation (26). We also found that it maps to the region of 11p15 LOH in breast cancer, near DllS860. In addition, the breast cancer gene BRCA1 has been reported to show abnormal cytoplasmic localization in breast cancer (29). Since NUP98 is a nucleoporin, it might be involved in a nuclear transport pathway involving BRCA1. For these reasons, we investigated the possibility that NUP98 is involved in breast cancer. The exon-intron boundaries were defined by sequencing of genomic clones using primers derived from the cDNA sequence. Primers derived from intronic sequences flanking exons were used to PCR amplify breast cancer samples showing LOH (and thus more likely to show mutations in the non-deleted allele). We have examined by single strand conformational polymorphism (SSCP)-PCR approximately 70% of the entire 3 kb coding region of the gene in 48 tumors, and we have detected one mutation in the gene, that changes a threonine to an alanine residue. This mutation was also present in the normal breast tissue of the patient, so we cannot exclude the possibility that it represents a polymorphism, albeit one involving a nonconservative amino acid substitution. We will attempt to obtain DNA from the patient's blood and from her parents, in order to determine whether the mutation could have arisen during embryonic development, or is a new germline mutation in the patient. We will also continue our mutational analysis of the remainder of the coding region of the gene. Our sequencing of NUP98 is shown in the Appendix:

The second candidate gene was $p57^{KIP2}$, a cyclin-dependent kinase inhibitor, which we mapped to the same approximate interval, approximately 1 Mb telomeric to NUP98 (18). We found that this gene undergoes genomic imprinting, with preferential expression of the maternal allele (27), as well as loss of imprinting (LOI) and epigenetic silencing in Wilms tumor (28). However, another laboratory has convincingly demonstrated that $p57^{KIP2}$ is not mutated in Wilms tumor (30), and is not responsible for suppression of tumorigenicity in that malignancy (31). One of our hypotheses is that there are multiple 11p15 tumor suppressor genes, and that they involve different malignancies.

We do not have data as yet regarding mutational analysis. However, we have begun to analyze $p57^{KIP2}$ expression using an RNase protection assay. Preliminary data on 6 tumors shows essentially no $p57^{KIP2}$ expression, compared to adjacent normal breast epithelium. We will extend this observation to approximately 30 samples. It is also critical to determine whether $p57^{KIP2}$ suppresses the growth of breast cancer cells when expressed at physiological levels. We have designed sense and antisense $p57^{KIP2}$ tetracycline-repressible expression constructs, that when electroporated into recipient cells, express $p57^{KIP2}$ at levels that vary from clone to clone, but are stable for a given clone, as has been described for similar constructs of other genes (32). Thus, we will be able to test whether restoration of expression of $p57^{KIP2}$ to the level found in normal breast epithelial cells suppresses tumor cell growth.

Task 5, Analysis of Loss of Imprinting (LOI) in Breast Cancer: As noted in the Introduction, this task was deleted, due to a reduction in budget at the time of the initial award, and because it was expected that funding would be received through an administrative supplement of the National Action Plan for Breast Cancer (NAPBC). Even though that supplement received an outstanding priority score (we were told it was among the top-rated proposals), it was not funded by the NAPBC. Since this work is of great potential significance to

the understanding and potential treatment of breast cancer, we continued work on it at a limited level, while continuing to pursue other avenues for its funding.

We originally discovered LOI in Wilms tumor (24), and this observation has subsequently been extended by our and other laboratories to a wide variety of both childhood and adult tumors (reviewed in ref. 33). We found that LOI involves a change in the epigenotype of the maternally inherited chromosome to a paternal pattern of gene expression. This results in both activation of the normally silent maternal allele of the insulin-like growth factor-II (IGF2) gene, and to repression of the normally expressed maternal allele of the H19 gene (23). As IGF2 is an important autocrine growth factor in breast cancer (13-17), and LOI causes a double dose of IGF2 (23), this could be an important pathway in tumor progression.

In addition, we also discovered that the gene for $p57^{KIP2}$, a cyclin-dependent kinase inhibitor that causes G_1 arrest, is localized to one region of LOH on 11p15 in breast cancer (18), and that it undergoes genomic imprinting, with preferential expression of the maternal allele (27). In work not supported by this grant, we have found that the $p57^{KIP2}$ gene undergoes abnormal imprinting and epigenetic silencing in Wilms tumor (23).

We had originally found that LOI of IGF2 occurs in some human breast cancers, although the frequency of this alteration was low (<25%). However, Cullen and others have shown that breast cancers show increased expression of IGF2 from both tumor and stromal cells (13-17). We therefore investigated the possibility that LOI may occur in stromal cells of breast cancers. Toward that end, we obtained 20 stromal cells lines derived from short-term culture of breast tumors, of which 6 were heterozygous for the transcribed Apa I polymorphism in IGF2, permitting analysis of imprinting. All 6 lines showed LOI of IGF2, increased IGF2 expression, and epigenetic silencing of H19, as shown in Fig. 4 below:

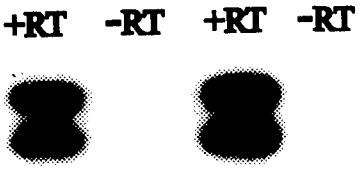

Fig. 4. Loss of imprinting of IGF2 in breast cancer. Lanes 1,3-breast cancer specimens, + RT, showing expression of 2 alleles. Lanes 2,4- – RT controls.

This is an important and surprising observation, because it indicates that an alteration in imprinting occurs in stromal cells of breast cancers, which have not been previously known to undergo genetic change. This alteration could explain the paracrine growth effect that breast tumor stromal cells exert on tumor cell growth.

Task 6, Analysis of Genes as Markers of Disease Subtype: This task is scheduled to commence after this reporting period (months 24-48).

3. Discussion

Over the past year, we have narrowed one region of LOH to the interval DllS4146 to TH, spanning approximately 2 Mb. We have molecularly cloned this region in genomic clones, and we have isolated 165 exons by exon trapping, as well as 24 genes within this interval to date. We have identified two genes that show alterations in other tumor types, NUP98 in leukemia, and $p57^{KIP2}$ in Wilms tumor. The latter change involves abnormal imprinting and epigenetic silencing rather than point mutation. We have analyzed both genes in breast cancer, and we have identified one missense mutation in NUP98, present in both normal and tumor DNA. We are currently determining whether this mutation is a germline mutation or a rare nonconservative transcribed polymorphism. Preliminary data also suggest that $p57^{KIP2}$ undergoes epigenetic silencing in breast cancer, similar to what we find in Wilms tumor.

We have also been able to overcome our earlier problems with genetic complementation experiments, designed to demonstrate directly the presence of a breast tumor suppressor gene within a defined genomic interval. These experiments are at the in vivo stage, and we should have meaningful data shortly. We have also prepared an expression construct that should permit physiological levels of $p57^{KIP2}$ expression, in order to determine whether the epigenetic silencing of this gene we see in breast cancer could play a causal role in breast cancer progression. This is a reasonable hypothesis, because LOH alone would cause silencing in the absence of mutation, since the gene is normally expressed almost entirely from the maternal allele. In addition, we have found that abnormal imprinting of this chromosomal region can also lead to altered expression of several genes, including $p57^{KIP2}$.

Finally, we have performed limited experiments directed at detecting a role for LOI of IGF2 in breast cancer progression. While we have not yet secured specific funding for these experiments, we felt that they were potentially too important to abandon. We have found that 6 of 6 breast tumor stromal lines, obtained from short term culture of breast cancer specimens, showed LOI of IGF2. This is an exciting observation, because it provides a molecular mechanism for mitogenic effects of breast cancer stromal cells, that have been long observed but little understood.

CONCLUSIONS

LOH experiments have defined a much smaller interval containing a breast cancer suppressor gene than we had mapped at the beginning of the study. Although it still represents a relatively large region (2 Mb), this is within our grasp for positional cloning. We have isolated exons at approximately 12 kb throughout this region, as well as 24 cDNA's to date corresponding to these exons. These reagents will be of tremendous value in identifying homozygous deletions, as well as candidate genes for breast cancer. We are also continuing our LOH experiments in the hope of refining this interval further, and also delimiting the more centromeric region of LOH more narrowly. These efforts will also be expedited by our approach of "microallelotyping" archival specimens that are more advanced than the majority of specimens of earlier stage that are generally available, given the data showing that 11p15 loss is specifically associated with breast cancer metastasis.

We have already identified two 11p15 candidate genes for breast cancer, NUP98 and $p57^{KIP2}$. The former shows a missense mutation leading to a nonconservative amino acid

substitution in one specimen, but we do not yet know whether this represents an early embryonic mutation, germline mutation, or rare polymorphism. Preliminary data also suggest that the imprinted gene $p57^{KIP2}$ undergoes epigenetic silencing in breast cancer. Our genetic complementation experiments using expression constructs of the gene will enable us to determine whether this gene is involved in breast cancer through the novel mechanism of genomic imprinting. We are also identifying additional candidate genes at a very rapid pace, and we are developing strategies for screening them much more quickly than was possible for us earlier.

Now that we have been able to propagate in vitro breast cancer cells containing STF's introduced into them by microcell transfer, we will also be able to delimit with a functional assay the regions containing breast cancer genes on 11p15. This work is important, because studies continue to show the biological relevance of one or more 11p15 genes to breast cancer progression. For example, 11p15 loss is specifically associated with lymph node metastasis, high S-phase fraction, and non-diploid and hormone receptor status (34).

Finally, although we are limited in funding for the study of imprinting in breast cancer, we have made the surprising and important discovery that breast cancers undergo loss of imprinting (LOI) of tumor stromal cells. These experiments have revealed a potential mechanism for the paracrine growth effects seen in these tumors, and they provide insight into the genetic basis for abnormal stromal cell function, about which very little is presently known. In other work, we have recently shown that it is possible to reverse LOI in tumors pharmacologically. Thus, the discovery of LOI in tumor stromal cells may lead to a novel chemotherapeutic strategy to the treatment of breast cancer.

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Appendix. Sequence of Human Nucleoporin Gene NUP98.

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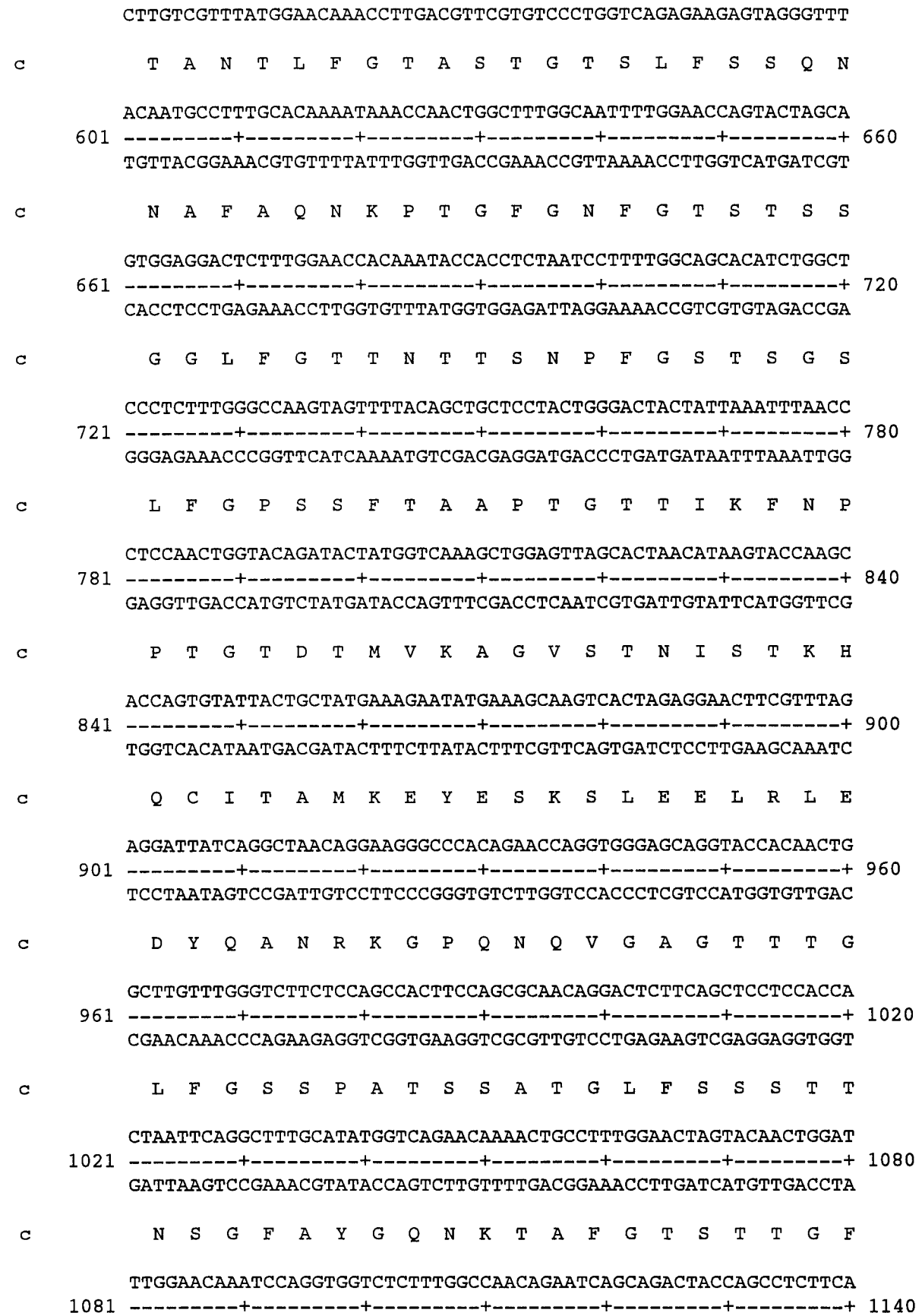
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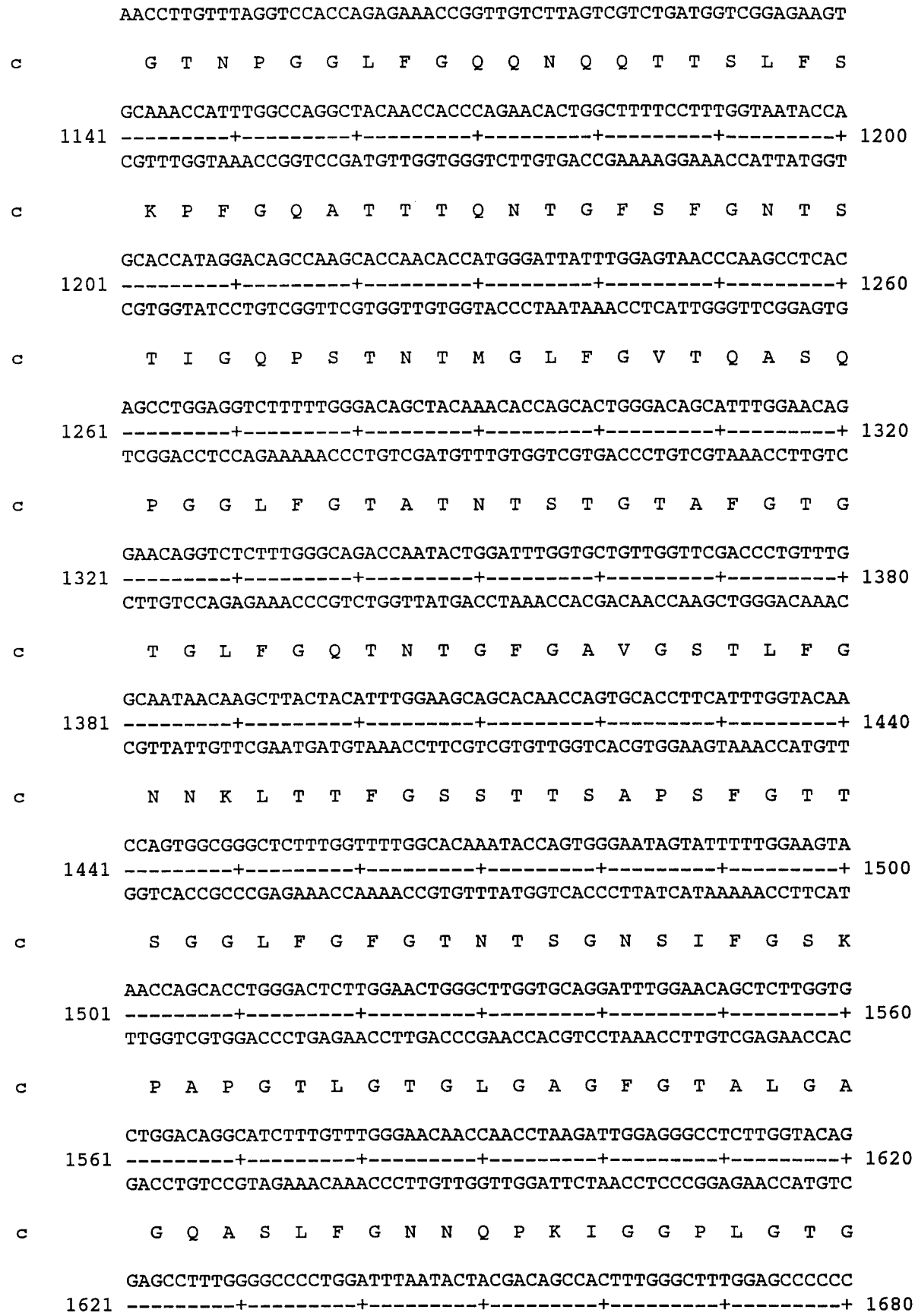
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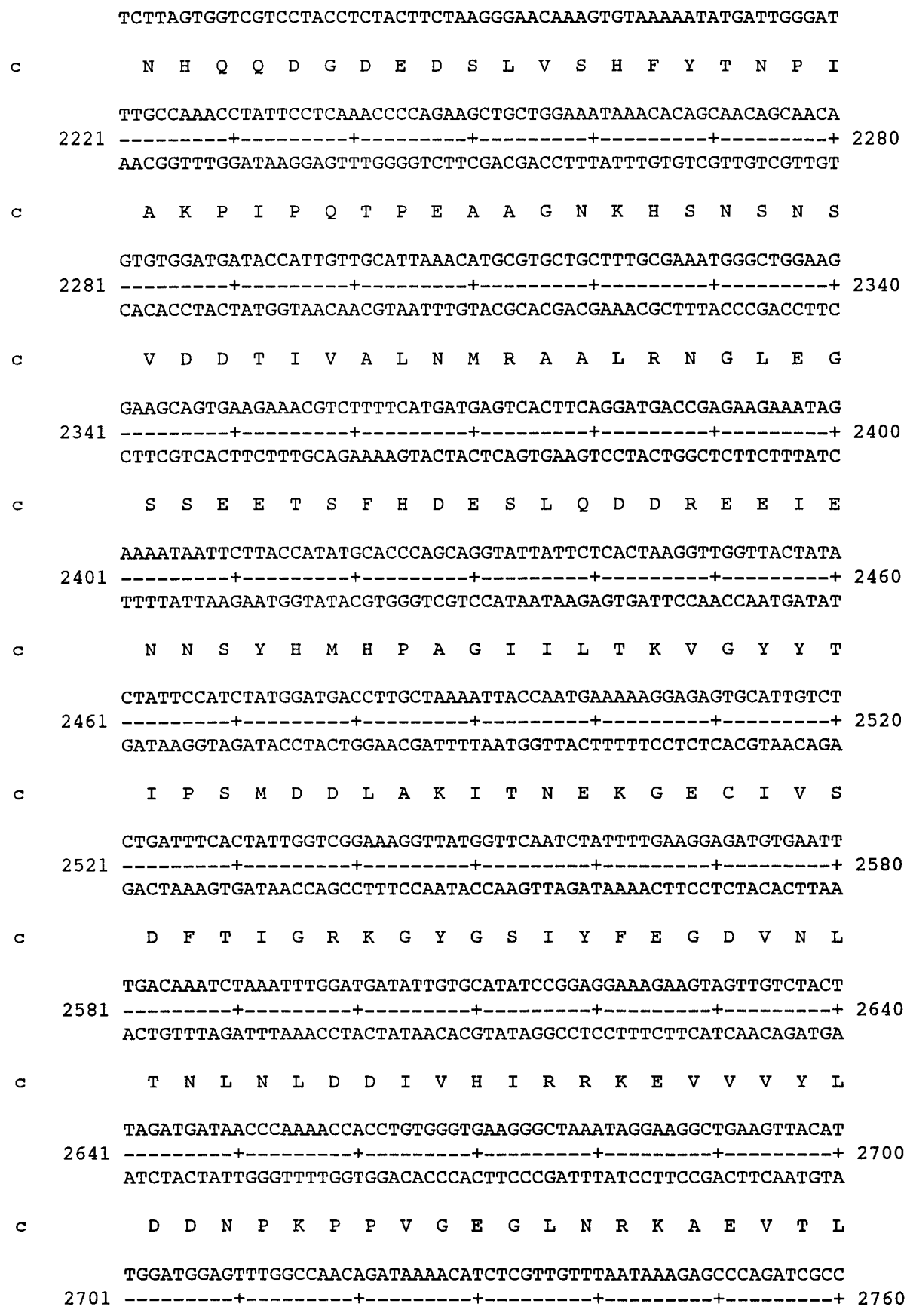
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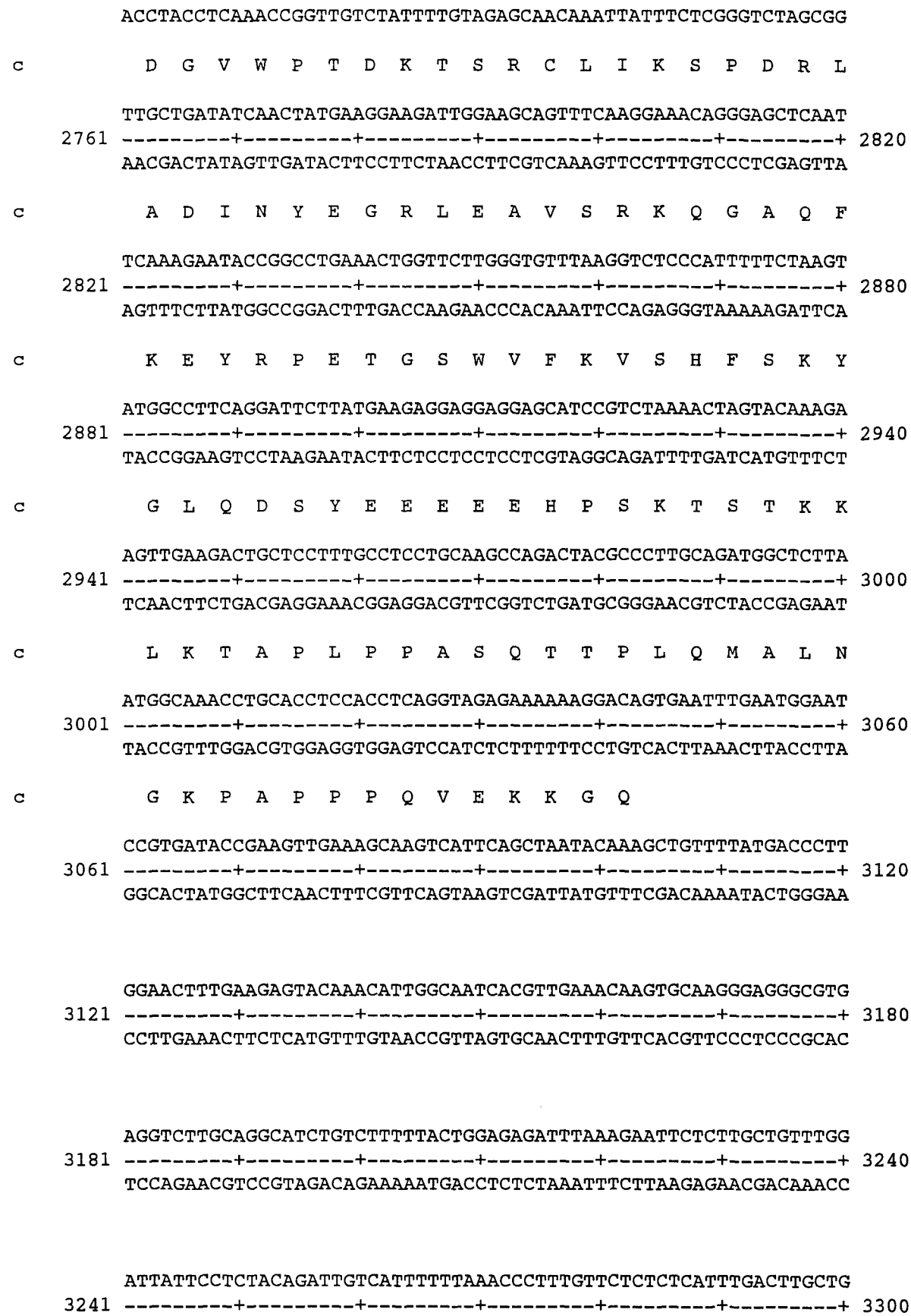
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     GTTAATGAACATAGTAACTATGCCCTAAAGAAACATTTCGTAGGAGATGGAGAGTCGACG


     TGAAAGCTAGACCTTTGGTATTTTCCATGCTATAATTCTTATGGCTGCTGAATGTGTGGT
3661 ---------+---------+---------+---------+---------+---------+ 3720
     ACTTTCGATCTGGAAACCATAAAAGGTACGATATTAAGAATACCGACGACTTACACACCA


     TTTTATGATTTATTAAATAATCTCTTAGGAGGCATTTCTGAAAAAAAAAAAAAAAAAAA
3721 ---------+---------+---------+---------+---------+---------+ 3780
     AAAATACTAAATAATTTATTAGAGAATCCTCCGTAAAGACTTTTTTTTTTTTTTTTTTT
```

Rec'd 7/19/2000

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 6 Jul 00

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statements

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the following awards:

DAMD17-94-C-4068	ADB218322
DAMD17-94-V-4036	ADB232944
DAMD17-94-J-4481	ADB222571
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DAMD17-96-1-6016	ADB228823
DAMD17-96-1-6073	ADB248567
DAMD17-94-J-4057	ADB221437, ADB247857
DAMD17-96-1-6069	ADB230256, ADB239319
DAMD17-95-1-5067	ADB236775, ADB249592
DAMD17-94-J-4308	ADB225776, ADB234457, ADB249935
DAMD17-96-1-6087	ADB232086, ADB238945, ADB250354
DAMD17-96-1-6075	ADB228777, ADB238338, ADB249653
DAMD17-95-1-5008	ADB225250, ADB236089, ADB243691
DAMD17-94-J-4310	ADB222453, ADB235860, ADB247801

Request the limited distribution statement for Accession Document Numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Virginia Miller at DSN 343-7327 or by email at Virginia.Miller@det.amedd.army.mil.

FOR THE COMMANDER:

Phyllis Rinehart

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management